P-283

PLASMID **39,** 1–9 (1998)
ARTICLE NO. PL971320

PERSONAL PERSPECTIVE

Plasmid (1952–1997)

Joshua Lederberg

Raymond and Beverly Sackler Foundation Scholar, The Rockefeller University, New York, New York 10021-6399

Received August 22, 1997; revised October 1, 1997

The term "plasmid" was introduced 45 years ago (J. Lederberg, 1952, *Physiol. Rev.* **32,** 403–430) as a generic term for any extrachromosomal genetic particle. It was intended to clarify the classification of agents that had been thought of disjunctively as parasites, symbionts, organelles, or genes. For a decade or more it was confused with "episome," although that was carefully crafted (F. Jacob and E. L. Wollman, 1958, *C. R. Acad. Sci.* **247,** 154–156) to mean agents with traffic in and out of chromosomes. Starting about 1970, plasmids became important reagents in molecular genetic research and biotechnology. They also play a cardinal role in the evolution of microbial resistance and of pathogenicity. The usage of the term has then escalated to its current peak of about 3000 published articles per year. The bedrock of genetic mechanism is no longer mitosis and meiosis of chromosomes; it is template-directed DNA assembly. This is often more readily studied and managed with the use of plasmids, which replicate autonomously outside the chromosomes. Some plasmids are also episomes, namely, they interact with the chromosomal genome, and other mobile elements may be transposed from one chromosomal locus to another without replicating autonomously. © 1998 Academic Press

It's a biological trick. . . . but something has transformed us from within, a plasmid has invaded our DNA, has twisted our nature. . . . (A. Wheelis, 1987, "The Doctor of Desire")

In its initial introduction (Lederberg, 1952), the term plasmid was defined as comprising any extrachromosomal genetic particle. It was intended to dissipate the controversy as to whether factors like *kappa* in *Paramecium, sigma* in *Drosophila,* the milk factor for mammary cancer and other vertically transmitted viruses in mice were "viruses" or "genes:" a dichotomy I felt to be false and mischievous. It seemed to me that (1) a gene might be either adaptive or dystonic in its impact upon the organism and (2) it was clearly evident that certain genes could be infectively transmitted from cell to cell (DNA-mediated transformation; virus-mediated transduction). Hence, neither physiological effect nor infective transmission should disqualify a particle from being part of the genotype of an organism. In the extreme, the same particle could be both gene and virus and with moderate change of physiology be seen as a symbiont or parasite.

> [This review, Cell Genetics and Hereditary Symbiosis,] stems, in part, from the discussions of cell genetics at the 'Golden Jubilee of Genetics' held at the Ohio State University September 1950 (Dunn, 1951). Much of this discussion centered on the primacy of the nucleus for the genetic determination of cell traits. Conflicting views were offered: Drosophila specialists tended to exclude extranuclear factors, whereas some students of microorganisms tended to emphasize them. If there is a difference, it may be due partly to the methodological peculiarities of the experimental materials, including the greater role of asexual reproduction in the life-history of microbes. Cytoplasmic inheritance aside, much of the interest in microbial genetics is focussed on the question of cell heredity. Similar questions have been raised by students of developmental biology, but differentiation in higher plants and animals afforded few opportunities for genetic analysis. (unpublished manuscript from an early draft of (J. Lederberg, 1952)

The context was a continuing "struggle for authority" in genetics between the "nuclear monopoly" and the advocates of the cyto-

0147-619X/98 $25.00
Copyright © 1998 by Academic Press
All rights of reproduction in any form reserved.

plasm (Sapp, 1987). One prevalent view was articulated by Loeb (1916) that

> the egg (or rather its cytoplasm) is the future embryo upon which the Mendelian factors in the chromosomes can impress only individual characteristics, probably by giving rise to special hormones or enzymes.

This was strenuously countered by Morgan (1926):

> Except for the rare cases of plastid inheritance all known characters can be sufficiently accounted for by the presence of genes in the chromosomes. In a word the cytoplasm may be ignored genetically.

We also have Muller (1951):

> In mitigation of the current conception that cytoplasmically located genes or gene-complexes form an essential part of the genetic constitution of animals, the following points should be noted: (1) the extreme rarity with which illustrations of such inheritance have been found in animal material, in contrast to the thousands of Mendelian differences found in them; (2) the dispensability of the cytoplasmically located particles in the cases studied and the absence of evidence of the existence of normal alternative forms of them; (3) the fact that, in these same cases, the agents have been proved to be able to pass as infections from one cell to another; and (4) the lack of a fundamental basis for distinguishing between these and cases of undoubtedly parasitic or symbiotic microorganisms or viruses of exogenous derivation.

and Beadle (1949):

> In view of the elaborate mechanisms of mitosis and meiosis, which have evolved and persisted throughout almost the whole of the plant and animal kingdom and which evidently have a great selective advantage, it would be most remarkable if the cytoplasm could compete as a carrier and transmitter of hereditary units in any except a few very special circumstances.

During the 1950s, there was a further tinge of political ideology that cytoplasmic inheritance might be associated with Lysenkist doctrine, which had criminalized the teaching of Mendelian genetics in the Soviet Union (Soyfer, 1994). It was lapped up by apologists for that doctrine, mostly among social rather than natural scientists and correspondingly attracted threatening attention from the Red-hunters of the era. What we now picture as anticommunist hysteria spilled over into the affairs of the Genetics Society of America; it took some persuasion on my part and others to avert the Society from adopting an anti-Lysenkist position as its own official doctrine (Genetics Society of America, 1950; Sapp, 1987). As a compromise, the Mendelian jubilee was to be the centerpiece of an open campaign of celebration (Dunn, 1951).

Sonneborn (1946) with his work on *kappa* in *Paramecium,* mounted a major challenge to the nuclear monopoly. His adversaries were then quite gleeful at the serial discoveries that *kappa* could be visualized, could be transmitted infectively, and eventually could be identified as a symbiotic bacterium, now named *Caedibacter* (Pond *et al.,* 1989). With regard to Lysenko, Sonneborn took great pains to dissociate himself from that abuse of his research and particularly from the sovereignty of the Communist Party in deciding a scientific controversy (Sonneborn, 1950).

My own focus in bacterial genetics had been pure Mendel–Morganism right down to linkage mapping (J. Lederberg, 1947)—perhaps to a fault. Nevertheless, I had the highest regard for Sonneborn, as a pioneer in the genetics of unicellulars and gratitude for the nurturing role he had played in my own career. In fact, in the early 1950s we had been actively discussing being collaborators on a monograph on the genetics of microorganisms. However, he played no active role in the production of this review.

I did feel that to dismiss a genetic particle as being merely a parasite was to overlook an important aspect of cell genetics and biology; so my mission was to bring the whole field of endosymbiosis into the consciousness of geneticists. Perhaps *Physiological Reviews* was not an ideal vehicle for this purpose; but its editor, Ralph Gerard at the University of Chicago had persuaded me of its prestige. At the time I was a 27-year-old associate professor at the University of Wisconsin. I doubtless met Gerard in Chicago at one of the frequent meetings of the "Midwest Phage Club" that Leo Szilard had organized to further his own education (Lanouette, 1992).

In addition to *Paramecium/kappa,* one of the most compelling stories of cytoplasmic

inheritance was the "petites colonies" phenomenon in yeast as told by Ephrussi *et al.* (1949). By 1952 it seemed most plausible that the PC phenotype was seated in mitochondria and that these could be cured (like chloroplasts in Euglena, kinetoplasts in trypanosomes, and, a few years later, F in *Escherichia coli*) by acridine dyes. The precise mechanism remains enigmatic. In 1957, in my only foray into yeast genetics, Bob Wright and I did formally demonstrate the cytoplasmic transmission of the PC+ trait, making cybrids via synkaryon formation before sporogenesis (Wright and Lederberg, 1957). "Curing" a yeast of its aerobic respiration did intrude a compelling convergence of chemotherapy with genetics, of symbiont with genes; in a word, it evoked a plasmid.

At the time of writing "Cell Genetics . . ." I was also informed by the initial experiments in my lab on *lambda* (E. M. Lederberg, 1951) and on the conjugal factor in *E. coli* K-12, namely F (Lederberg *et al.*, 1952). These were not far enough along to provide strong factual support to the plasmid concept, and I would still label that as hypothetical a few years further. *Lambda* had been named as a follow on to *kappa* (and for lysogen vs killer phenotypes). Our first assumption was that in the lysogenic state, *lambda* was an intracellular plasmid; but we soon learned that lysogeny segregated in crosses in close linkage with *gal,* that it behaved as a chromosomal marker. Later, Ikeda and Tomizawa (1968) discovered that phage P1 exhibited the "type of lysogeny in which a prophage exists as a plasmid." Ironically, this was the a priori expectation we had had for *lambda* in 1951, falsified by the linkage experiments. As to F, we understood very little about its genetics that stage, other than its rapid infective propagation in a culture and the requirement for cell-to-cell contact.

The agnosticism of the Delbrück (1946) faction notwithstanding, I had long been impressed by Burnet's accounts of lysogeny and his prescient interpretations:

> . . . [re] the current controversy on the intimate nature of phage, whether it is an independent parasite or a pathologically altered constitutent of normal bacteria. In our view both these contentions have been completely proved, and . . . regarding them as irreconcilable alternatives is quite unjustified. . . . the usage being determined wholly by its functional activity at the time." (Burnet and McKie, 1929a)

Their report of pigment changes in lysogenized (?) staphylococci (Burnet and McKie, 1929b) was a forerunner of lysogenic conversion some years before (Freeman, 1951) had demonstrated it for toxin production in *Corynebacterium diphtheriae.* Similar ideas had been voiced by the Wollmans in 1925 (Galperin, 1987).

Also very much in mind was the shocking discovery (Zinder and Lederberg, 1952) that *Salmonella* phages could transduce chromosomal fragments from one bacterium to another. Judging from the displacement of old by newly inserted genes (streptomycin resistance, antigens) we inferred that these virus-borne genes had reentered the host chromosome. This had some analogical corroboration later from the specialized transduction of the *gal* segment by *lambda* (Morse *et al.*, 1956). Hence, as striking examples as these were of "infective heredity" in bacteria, only the F factors persisted as prototypic plasmids. Infective heredity might target the cytoplasm, the chromosome, or both seriatim. Until F was pinpointed as a satellite DNA (Marmur *et al.*, 1961) these interpretations remained highly conjectural.

The more compelling plasmid stories were of endosymbioses, and I discovered a rich lore in Buchner's *opus magnum* (1930). Over 700 pages recount every imaginable association, including provocative examples of transovarial transmission of microscopically visible symbionts. Were these symbionts invisible or obscured, they might well have been discovered as examples of cytoplasmic inheritance, in many cases providing vitally essential nutrients to the host.

From some of Buchner's colleagues, I learned that a new edition was forthcoming, and I acquired an early copy of "Endosymbiose der Tiere mit pflanzliche Mikroorganismen" (Buchner, 1953), but not in time for the review. I was determined to make this

work more readily available to English-speaking scientists, and eventually inveigled Edward A. Steinhaus and Walter Carter to do most of the work in locating a publisher and a competent translator. Finally, this was Bertha Mueller, one of Carter's colleagues at the University of Hawaii. As several publishers (who turned me down) insisted that this would have to be a labor of love, and we are certainly in her debt. Buchner's "Endosymbiosis of Animals with Plant Microorganisms" appeared in 1965. No single-authored work of that kind is likely to appear again (but see Margulis, 1993).

Even in Buchner's 1953 edition, Sonneborn's work with *Paramecium* is not mentioned—Buchner was somewhat impatient with genetic speculations complicating the presentation of the symbiotic way of life/lives.

The most comprehensive precedent for plasmids was probably Darlington's plasmagenes (1944), and this term might have been a reasonable contender. However, it had become confounded with Spiegelman's (1946) plasmagene theory of gene expression. This supposed (in anachronistic modern terminology) that messenger RNA was self-replicating and could allow for persistence of a phenotype beyond the presence of the initial chromogene. This highly plausible speculation has yet to find an authentic example. In addition, a plasmagene has connotations of simplicity; a plasmid might comprise scores or thousands of genes. Plasmosome would be a counterpart to chromosome; but the term was preempted as a synonym of nucleolus. It was important to have a new term, bereft of confusing baggage. As perceptively epitomized by Sapp (1994):

> Lederberg's plasmid was by no means an impartial term introduced to neutralize sometimes conflicting connotations of the others. It implied that all extranuclear entities would have to be treated equally as genetic constituents of cells, regardless of their origin or function. It was an argument that allowed for agnosticism with regard to the origins of intracellular constituents. It was a discursive maneuver that worked in two directions: a self-reproducing cytoplasmic entity of exogenous origin could and should be treated as part of the genetic constitution of the cell, and therefore (in reverse) any part of the genetic constitution of the cell cytoplasm might be of exogenous origin. A particle shown to be infectious would have to be treated as if it were not; a particle not shown to be infectious would be treated as if it once might have been.

The term plasmid was then invented as a hybrid of cytoplasm or plasmagene and "-id", as in plastid, chromatid, or id (Weissmann, Freud, Latin "it"). Insistence that it might apply to any extrachromosomal particle may leave some uneasy, and it is rarely applied nowadays to mitochondria or chloroplasts which have a firmly established identity; but the logic of that assimilation has been consensually accepted—at least to the point of reasonable debates about the boundaries of the organism (Russert-Kraemer and Bock, 1989). We now have talk of the converse, of selfish DNA, whereby the persistence and expansion of some chromosomal parts betokens a parasitic function (Fig. 1).

PLASMID SINCE 1952: CRYPTIC FROM 1952 TO 1963

Further work from our laboratories (Lederberg and Lederberg, 1953) and the Paris group (Jacob and Wollman, 1957) substantiated that a virus like *lambda,* as part of its life cycle, might also go in and out of the chromosome, the duality that supported another term, episome, introduced by Jacob and Wollman (1958). For a time "episome" dominated the discussion of extrachromosomal agents, often in contexts that ignored or even violated the condition of chromosomal habitat. For a decade from 1952, the term plasmid was scarcely used in experimental reports; it appears in the title of none of my own scientific papers. The earliest title I have been able to locate is Coe (1961). In 1963, Novick (1963) remarked, tentatively.

> If, indeed, an extrachromosomal particle is involved in penicillinase inheritance, in the absence of evidence regarding reversible attachment to the chromosome, the term 'plasmid' (Lederberg, J., 1952) will be preferable to 'episome' (Jacob and Wollman, 1958) in describing it.

Then Stocker and Dubnau (1964) refer to

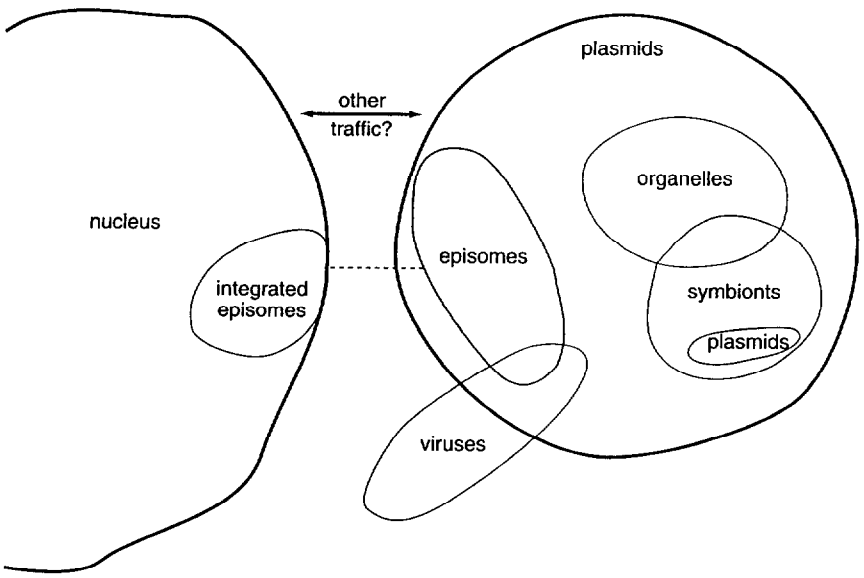

FIG. 1. Venn diagram of the endocellular world: the logical categories, some inclusive of one another, or with overlapping boundaries, of various endocellular genetic agents. Plasmids are not logically a subset of symbionts, but components of symbionts may be plasmids. The void between "nucleus" and "plasmid" may be occupied by other entities or processes that lack essential properties of a genetic particle.

"Genetics of plasmids in *Salmonella typhimurium*": that may have been influenced by Stocker's stay in my laboratory in 1952–1953. This was followed by Richmond (1965), "Penicillinase plasmids in *Staphylococcus aureus*," who remarked that

> there may be some differences between these plasmids and true episomes . . . no evidence that the penicillinase genes can ever exist as an integral part of the chromosome of *Staph. aureus*.

In the late 1950s, several Japanese researchers had uncovered the RTFs, or resistance transfer factors, in gram-negative bacteria (Watanabe, 1963). For some time these were classified as episomes; but with a paucity of evidence for chromosomal integration, the term plasmid began to take hold. Together with colicinogenic factors that, likewise, became identified with the ability to mobilize their own conjugal transfer apart from F, these plasmids provided molecular geneticists with marvelous new tools for manipulation as well as analysis. That association has led to a usage whereby plasmid is often taken to refer to that specific category of circular DNA in bacteria. A climactic event was the reconstruction of an intergeneric (*Staph.* × *E. coli*) plasmid by DNA-splicing technology (Chang and Cohen, 1974). Since then "plasmid" has been emblematic of biotechnology, ranging from the most fundamental studies to applications in industry and in the clinic. The explosion of publications about plasmids has mostly to do with their uses and great concern about the advantages of promiscuous gene exchange to pathogenic bacteria in their evolutionary competition (Sonea and Panisset, 1983; Chadwick and Goode, 1997). Many authors may mention particular plasmids, like pSC101 or pBR322, by their given names, and see no need to refer more broadly to the term plasmid nor the conception behind it: this is a pale shadow of usage with regard to "gene" or "virus." Most of the fundamental issues about extrachromosomal heredity that so exercised the pioneers of Mendelian genetics, and which motivated the 1952 review, are now taken for granted.

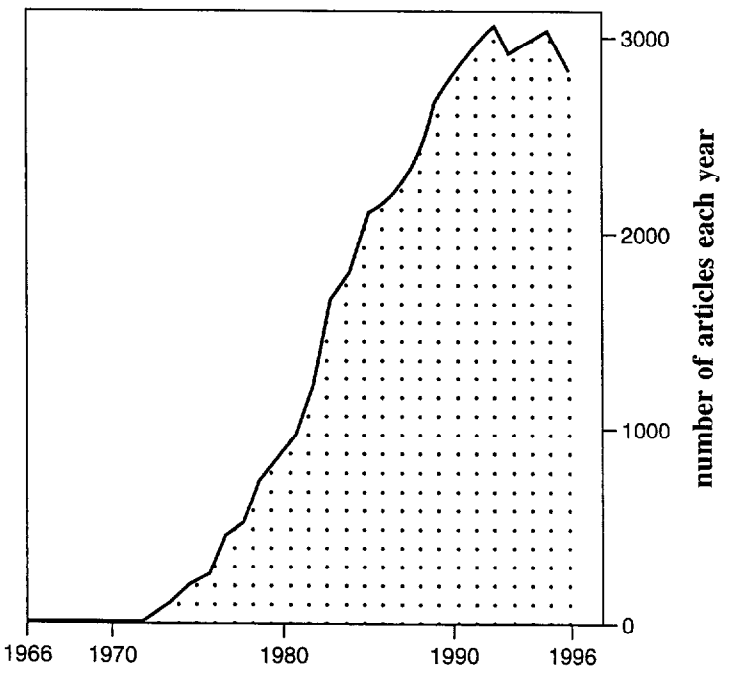

FIG. 2. Items referenced by Medline in indicated years, 1966–1996. The abscissa is year by year; the ordinate number of publications in a given year. These figures are influenced by MEDLINE's journal coverage and by limited availability of abstracts in the first decade. The figures from 1963 to 1973 are less than 20 per year and are invisible on this scale. "Plasmid.tw" refers to the medline code for using *plasmid* as a text-word key.

Today, according to MEDLINE (Fig. 2) and to the Science Citation Index, about 3000 articles a year are published with "plasmid" in the title or abstract. This constitutes about 0.5% of that database, compared, say, with 5% for "DNA." Some web browsers will report from 10,000 to 40,000 hits. At least one discussion list is devoted to "plasmids"; contact esfplasnet-owner@bham.ac.uk for further details.

SYMBIONTS AS PLASMIDS AS ORGANELLES

In a more general biological sphere, however, there has been a marked revival of interest in endocytobiology (Lee and Frederick, 1987) particularly as more scientists have perceived how this bears on the definition of the individual organism (Russert-Kraemer and Bock, 1989; Buss, 1987). Hyperparasites or coenocytes admitting of the intermingling of nonhomologous nuclei within a common cytoplasm illustrate that conceptual challenge. (Wostemeyer *et al.,* 1995).

Some Definitional Problems

Given that a plasmid is an extrachromosomal symbiont, tacitly within the cell membrane, we must consider the boundaries both of chromosome and of cell. Sharper definitions are perhaps needed. We could, for example, consider the mitochondrium as a multicopy 24th chromosome. But it is not partitioned and segregated on the mitotic spindle, and let us decide to leave that as the boundary.

If one insists on plasmids as small, dispensable, circular, double-stranded DNA, as some have done, we would have to find additional categories for noncircular plasmids and for RNA plasmids. The criterion of dispens-

ability is highly context dependent; humans would not fare very well deprived of aerobic metabolism. And bacteria may find their needs for nutrition or toxin resistance met with equal grace by nuclear or plasmidic genes. Not to mention how "dispensable" are the majority of chromosomal genes, and beyond that how much of our own chromosomes are junk DNA.

In 1952, we knew that chloroplasts were endowed with genetic continuity and surmised the same for mitochondria. The analogy of chloroplasts with endosymbiotic algae in protozoa was particularly compelling. These entities would then also fall within the category of plasmids. Perhaps, like other plasmids, they were also endosymbionts and might even be cured by environmental exposure as indeed proves to be the case. Such ideas had been sponsored by Altmann and Mereschkovskii many years before and they received sympathetic attention in my review, though I hardly credited Wallin's claims of having cultivated the mitochondria *in vitro.* Thanks largely to the clear insight, articulation, and perserverance of Lynn Margulis, and supported by experimental dissection of mitochondrial and plastid DNA, the symbiotic origin of these particles is now universally accepted. She has told how that 1952 review gave her early encouragement for her pursuit (Margulis, 1993).

As we know, "smaller fleas . . . have smaller still to bite 'em" (Swift, 1712), and we should not be startled that organelles, symbionts, in that sense plasmids, may have "smaller still to bite 'em." They have been found to inhabit *Paramecium's kappa* particles and may account for their toxin production.

PLASMID: CONCEPT OR TERMINOLOGY

As history has taught us with regard to the gene (Carlson, 1966; Falk, 1984), the concept of plasmid is more important than a probably futile effort to police its usage. The cell is a consensually accepted unit of biological structure. So far all cells (pace nonviable minicells) envelop a genetic core readily apprised as a nucleus or nucleoid and consisting of one more chromosomes. In addition, most cells are adorned with a further set of hereditary determinants (plasmids *sensu latu*) recognizably distinct from, usually much smaller than, the nucleus/chromosome. They may vary in origin, persistency, and fate, and we may often be ignorant—even agnostic—about these parameters. With traffic in and out of the chromosomes, they include episomes and orthogonally may be labeled as viruses, mitochondria, chloroplasts, and a whole array of endosymbionts and parasites. Today, DNA (or RNA) is the common thread that unites them. "Plasmid" is a reminder that not all the nucleic acid is in the nucleus/nucleoid.

Any scheme that purports to span all of life will have complications.

- Where do we place prions?
- Where do we classify double minutes, accessory chromosomes, BACs, and YACs especially as some drift away from the strict regulation of the mitotic mechanism.
- A chromosomal genome can be naturally or artificially dissected into two or more pieces. If we call one the chromosome—an arbitrary decision—the other(s) become a plasmid (Kolsto, 1997; Itaya and Tanaka, 1997).
- Episome or not: this is often regulated by sequence homology, now manipulable by genetic engineering.
- A host of systems of genetic versatility: replicons, transposons, integrons . . .
- Heterokaryons tell us that a cell membrane may envelop a complex community of nuclear domains.
- Genomes interact via gene products across those envelopes.
- New categories of plasmids (ecc-DNAs) may occur or be newly generated in mammalian cells as well as other habitats (Gaubatz, 1990).
- We do not forget the small circular DNAs which are the bread and butter of the plasmid industry.

The editor of this journal has outlined the broadening concepts of genetic mobility

which are an appropriate fit for *Plasmid* (Macrina, 1997); some of these were already anticipated at the founding (Novick, 1977).

Since 1952, molecular genetics has been overtaken by the DNA revolution; the nuclear (chromosomal) monopoly is supplanted by the realm of the nucleic acids. We are no longer impelled to make such sharp distinctions between DNAs based on geography, and the bedrock of genetic mechanism is no longer mitosis and meiosis; it is template-directed DNA assembly. The question "where is the cytoplasm?" devolves into a search for epi- and extranucleic modes of genetic determination (Lederberg, J., 1958), especially in differentiation and somatic cell inheritance. That is, heritable distinctions superimposed on an informationally conserved DNA sequence. And we have learned to be cautious about customary dogma that had excluded nucleic mechanisms from any part of epigenesis, namely, development. We know the contrary, at least for the immune system (J. Lederberg, 1988). Conversely, the decks are now cleared for a fresh foray beyond DNA in the continuity of some cellular functions (Sapp, 1997).

REFERENCES[1]

Beadle, G. (1949). Genes and Biological Enigmas. *In* "Science in Progress" (G. A. Gaitsell, Ed), pp. 184–249. Yale Univ. Press, New Haven, CT.

Buchner, P. (1930). "Tier und Pflanze in Symbiose." Borntraeger, Berlin.

Buchner, P. (1953). "Endosymbiose der Tiere mit pfanzlichen Mikro-organismen." Verlag Birkhausen, Basel.

Buchner, P. (1965). "Endosymbiosis of Animals with Plant Microorganisms." Interscience, New York.

Burnet, F. M., and McKie, M. (1929a). Observations on a permanently lysogenic strain of *B. enteritidis* Gaertner. *Austr. J. Exp. Med. Sci.* **6,** 277–284.

Burnet, F. M., and McKie, M. (1929b). Type differences among staphylococcal bacteriophages. *Austr. J. Exp. Med. Sci.* **6,** 21–31.

Buss, L. W. (1987). "The Evolution of Individuality." Princeton Univ. Press, Princeton, NJ.

Carlson, E. A. (1966). "The Gene: A Critical History." Saunders, Philadelphia, PA.

Chadwick, D. J., and Goode, J. (Eds.) (1997). "Antibiotic Resistance: Origins, Evolution, Selection and Spread." Ciba Foundation Symposium, Vol. 207. Wiley, Chichester.

Chang, A. C., and Cohen, S. N. (1974). Genome construction between bacterial species in vitro: Replication and expression of *Staphylococcus* plasmid genes in *Escherichia coli. Proc. Natl. Acad. Sci. USA* **71,** 1030–1034.

Clark, A. J., and Adelberg, E. A. (1962). Bacterial conjugation. *Ann. Rev. Microbiol.* **16,** 289–319.

Coe, E. H., Jr. (1961). Some observations bearing on plasmid versus gene hypotheses for a conversion-type phenomenon. *Genetics* **46,** 719–725.

Cohen, S. N. (1993). Bacterial plasmids: Their extraordinary contribution to molecular genetics. *Gene* **135,** 67–76.

Darlington, C. (1944). Heredity, development and infection. *Nature* **154,** 164–169.

Delbrück, M. (1946). Bacterial viruses or bacteriophages. *Biol. Rev.* **21,** 30–40.

Driskell-Zamenhof, P. (1964). Bacterial episomes. *In* "The Bacteria" (I. C. Gunsalus and R. Y. Stanier, Eds.), Vol. V, pp. 155–222. Academic Press, New York.

Dunn, L. C. (Ed.) (1951). "Genetics in the 20th Century." Macmillan, New York.

Ephrussi, B., Hottinguer, H., and Chimenes, A. M. (1949). Action de L'Acriflavine sur les Levures. I. La Mutation Petite Colonie. *Ann. Inst. Pasteur* **76,** 351–367.

Falk, R. (1984). The gene in search of an identity. *Human Genet.* **68**(3), 195–204.

Freeman, V. J. (1951). Studies on the virulence of bacteriophage-infected strains of *Corynebacterium diphtheriae. J. Bacteriol.* **61,** 675–688.

Galperin, C. (1987). Le bactériophage, la lysogénie et son déterminisme génétique. *Hist. Phil. Life Sci.* **9,** 175–224.

Gaubatz, J. W. (1990). Extrachromosomal circular DNAs and genomic sequence plasticity in eukaryotic cells. *Rev. Mutat. Res.* **237**(5–6), 271–292.

Genetics Society of America (1950). Records, Number 19. Published by the Secretary.

Ikeda, H., and Tomizawa, J. (1968). Prophage P1, an extrachromosomal replication unit. *Cold Spring Harbor Symp. Quant. Biol.* **33,** 791–798.

Itaya, M., and Tanaka, T. (1997). Experimental surgery to create subgenomes of *Bacillus subtilis* 168. *Proc. Natl. Acad. Sci. USA* **94,** 5378–5382.

Jacob, F., and Wollman, E. L. (1957). Genetic aspects of lysogeny. *In* "Symposium on The Chemical Basis of Heredity" (W. D. McElroy and B. Glass, Eds.), pp. 468–498. Johns Hopkins Press, Baltimore.

Jacob, F., and Wollman, E. L. (1958). Les épisomes, élé-

[1] *Bibliographic note.* Narrative undocumented herein about the history of plasmid research can be substantiated in the following reviews: Clark and Adelberg (1962), Marmur *et al.* (1963), Driskell-Zamenhof (1964), Cohen (1993).

ments génétiques ajoutés. *C. R. Acad. Sci.* **247,** 154–156.

Kolsto, A. B. (1997). Dynamic bacterial genome organization. *Mol. Microbiol.* **24,** 241–248.

Lanouette, W. (1992). "Genius in the Shadows: A Biography of Leo Szilard." Scribners, New York.

Lederberg, E. M. (1951). Lysogenicity in *E. coli* K-12. *Genetics* **36,** 560.

Lederberg, E. M., and Lederberg, J. (1953). Genetic studies of lysogenicity in *Escherichia coli. Genetics* **38,** 51–64.

Lederberg, J. (1947). Gene recombination and linked segregations in *Escherichia coli. Genetics* **32,** 505–525.

Lederberg, J. (1952). Cell genetics and hereditary symbiosis. *Physiol. Rev.* **32,** 403–430.

Lederberg, J. (1958). Genetic approaches to somatic cell variation: Summary comment. *J. Cell. Comp. Physiol.* **52**(Suppl. 1), 383–402.

Lederberg, J. (1988). The ontogeny of the clonal selection theory of antibody formation: Reflections on Darwin and Ehrlich. *Ann. N. Y. Acad. Sci.* **546,** 175–187.

Lederberg, J., Cavalli-Sforza, L. L., and Lederberg, E. M. (1952). Sex compatibility in *Escherichia coli. Genetics* **37,** 720–730.

Lee, J. J., and Frederick, J. F. (Eds.) (1987). International Colloquium on Endocytobiology, 3rd, 1986. *Annals of the N. Y. Acad. Sci.* 503.

Loeb, J. (1916). "The Organism as a Whole." Putnam, New York.

Macrina, F. L. (1997). Plasmid at 20 years. *Plasmid* **37**(1), 1.

Margulis, L. (1993). "Symbiosis in Cell Evolution. Microbial Communities in the Archean and Proterozoic Eons." Freeman, New York.

Marmur, J., Rownd, R., Falkow, S., Baron, L. S., Schildkraut, C., and Doty, P. (1961). The nature of intergeneric episomal infection. *Proc. Natl. Acad. Sci. USA* **47,** 972–979.

Marmur, J., Falkow, S., and Mandel, M. (1963). New approaches to bacterial taxonomy. *Ann. Rev. Microbiol.* **17,** 329–372.

Morgan, T. H. (1926). Genetics and the physiology of development. *Am. Nat.* **60,** 489–515.

Morse, M. L., Lederberg, E. M., and Lederberg, J. (1956). Transduction in *Escherichia coli* K-12. *Genetics* **41,** 142–156.

Muller, H. J. (1951). The development of the gene theory. *In* "Genetics in the 20th Century" (L. C. Dunn, Ed.), pp. 77–100. Macmillan, New York.

Novick, R. P. (1963). Analysis by transduction of mutations affecting Pencillinase formation in *Staphylococcus aureus. J. Gen. Microbiol.* **33,** 121–136.

Novick, R. P. (1977). Editorial: On the launching of *Plasmid. Plasmid* **1,** iii–iv.

Pond, F. R., Gibson, I., Lalucat, J., and Quackenbush, R. L. (1989). R-body-producing bacteria. *Microbiol. Rev.* **53**(1), 25–67.

Richmond, M. H. (1965). Penicillinase plasmids in *Staphylococcus aureus. Br. Med. Bull.* **21**(3), 260–263.

Russert-Kraemer, L., and Bock, W. J. (1989). Prologue—The necessity of the organism. *Am. Zool.* **29**(3), 1057–1060.

Sapp, J. (1987). "Beyond the Gene: Cytoplasmic Inheritance and the Struggle for Authority in Genetics." Oxford Univ. Press, Oxford.

Sapp, J. (1994). "Evolution by Association: A History of Symbiosis." Oxford Univ. Press, New York.

Sapp, J. (1997). Cytoplasmic heretics. *Perspect. Biol. Med.*, in press.

Sonea, S., and Panisset, M. (1983). "A New Bacteriology." Jones and Bartlett, Boston, MA.

Sonneborn, T. M. (1946). Experimental control of the concentration of cytoplasmic genetic factors in *Paramecium. Cold Spring Harbor Symp. Quant. Biol.* **11,** 236–255.

Sonneborn, T. M. (1950). Heredity, environment and politics. *Science* **111,** 529–539.

Soyfer, V. N. (1994). "Lysenko and the Tragedy of Soviet Science." Rutgers Univ. Press, New Brunswick, NJ.

Spiegelman, S. (1946). Nuclear and cytoplasmic factors controlling enzymatic constitution. *Cold Spring Harbor Symp. Quant. Biol.* **11,** 256–277.

Stocker, B. A. D., and Dubnau, D. A. (1964). Genetics of plasmids in *Salmonella typhimurium. Nature* **204,** 1112–1113.

Swift, J. (1712). On Poetry. *In* "A New Dictionary of Quotations on Historical Principles from Ancient and Modern Sources." (1942). (H. L. Mencken, Ed.). Knopf, New York.

Watanabe, T. (1963). Infective heredity of multiple drug resistance in bacteria. *Bacteriol. Rev.* **27,** 87–115.

Wheelis, A. (1987). "The Doctor of Desire." Norton, New York.

Wostemeyer, J., Wostemeyer, A., Burmester, A., and Czempinski, K. (1995). Relationships between sexual processes and parasitic interactions in the host–pathogen system *Absidia glauca–Parasitella parasitica. Can. J. Bot.* **73**(S1A-), S243–S250.

Wright, R. E., and Lederberg, J. (1957). Extranuclear transmission in yeast heterokaryons. *Proc. Natl. Acad. Sci. USA* **43,** 919–923.

Zinder, N. D., and Lederberg, J. (1952). Genetic exchange in *Salmonella. J. Bacteriol.* **64,** 679–699.

Communicated by F. L. Macrina